Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG 4 Appendix 2.04

Title: Flow Chart Specific for FSIS Laboratory Isolation and Identification of Salmonella

Effective Date: 1/02/19

Description and purpose of change(s):

This flow chart describes FSIS laboratory testing included in MLG 4 Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Egg, and Siluriformes (Fish) Products and Carcass and Environmental Sponges
Flow Chart Specific for FSIS Laboratory Isolation and Identification of *Salmonella*

**PRIMARY ENRICHMENT**

- **Day 1:** Various Samples + Enrichment
  - Incubate BPW @35°C for 18-24 hrs (RTE), 20-24 hrs (raw poultry) or 22-26 hrs (fish).
  - Incubate mTSB @42°C for 15-24 hrs (raw meat).
  - NOTE: Sample receipt temperature of ≤15°C is required for raw beef products.

**SECONDARY (SELECTIVE) ENRICHMENT**

- **Day 2:**
  - Perform 3M™ Molecular Detection Assay 2 - *Salmonella* ²
  - (+) or (-)**

- **Day 3:**
  - Transfer 0.5 ml of enrichment broth to 10ml TT³ tubes
  - Transfer 0.1 ml of enrichment broth to 10ml RV⁴ tubes
  - Incubate all tubes @42°C for 22-24 hrs

- **Day 4:**
  - Streak tubes of RV and TT to BGS⁵ and DMLIA⁴ plates.
  - Incubate @35°C for 18-24 hrs.

- **Day 5:**
  - Re-incubate all BGS and DMLIA plates @35°C for 18-24 hrs.
  - Pick typical colonies (T), stab and streak TSI¹ / LIA⁸ slants.
  - Incubate @35°C for 22-26 hrs.
  - Optional: also streak SBA⁹ plate now.
  - Incubate @35°C for 16-24 hrs

- **Day 6:**
  - NT or (-)

**DIFFERENTIAL & SELECTIVE ISOLATION MEDIA**

- **Day 6:**
  - Transfer growth from TSI or LIA slants to SBA⁹ plate, streak for purity
  - Incubate @35°C for 16-24 hrs

**SUGAR FERMENTATION & H₂S/GAS PRODUCTION**

- **Day 7:**
  - Examine SBA growth for purity
  - Prepare saline suspension
  - Test on VITEK²³
  - (+) or (-)**

**BIOCHEMICAL IDENTIFICATION**

- **Day 6:** (Vitek² result may be Day 7)
  - Prepare saline suspension
  - Test on VITEK²³
  - (+) or (-)**

**CONFIRMED POSITIVE ISOLATE**

- FSIS Labs: Pick isolates to nutrient agar deep for further characterization testing and molecular serology at EL-MCB¹¹
- Pick isolates to cryobeads for storage.

**EXAMINATION FOR TYPICAL REACTIONS**

- PRESUMPTIVE POSITIVE REPORTED EXCEPT FOR HACCP
- ****Negatives reported

**PURITY/GROWTH**

- ****This chart represents the best-case scenario but analyses may take longer due to analytical testing circumstances such as restreaking isolates for purity or one day shorter if Day 4 option is followed.

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1. BPW = Buffered Peptone Water
2. 3M™ Molecular Detection Assay = Rapid Screen Test
3. TT = TT broth (Hajna)
4. RV = Rappaport-Vassiliadis R10 Broth
5. BGS = Brilliant Green Sulfadiazin Agar
6. DMLIA = Double Modified Lysine Iron Agar
7. TSI = Triple Sugar Iron Agar
8. LIA = Lysine Iron Agar
9. SBA = Sheep Blood Agar
10. VITEK® 2 = VITEK® 2 Compact System Automated Biochemical Identification System
11. Eastern Laboratory Microbiology Characterization Branch (EL-MCB), USDA, FSIS
12. Modified Typtone Soya Broth

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Effective: 1/02/19
Issuing Authority: Director, Laboratory Quality Assurance Staff (LQAS)